To,

Prof. Arnab Halder,

Head Department of Chemistry,

Presidency University, Date:07.06.2022

Kolkata 73

Respected Sir,

This is to inform you that I, Prasenjit Mondal, a research fellow from the Department of Chemistry, Presidency University, have completed the two-year course after the registration (R-17RS205120098), held on 10th July, 2018, under the supervision of Dr. Adity Bose. I would like to submit my 5000-word paper titled "INVESTIGATION ON THE INTERACTION OF DIETARY PHENOLIC ACID AND THEIR DERIVATIVES WITH BIOLOGICALLY SIGNIFICANT MOLECULES: A MULTI SPECTROSCOPIC ANALYSIS". According to the University's guidelines, I have also met all of the required criteria.

Please see the attachment, which contains all scanned papers, including coursework mark sheets, registration certificates, conference certificates, and publications, as well as my 5000 words.

I would like to request you to do the needful in submitting my 5000-word thesis.

Thanking You.

Yours Sincerely

Prasenjit Mondal

INVESTIGATION ON THE INTERACTION OF DIETARY PHENOLIC ACID AND THEIR DERIVATIVES WITH BIOLOGICALLY SIGNIFICANT MOLECULES: A MULTI SPECTROSCOPIC ANALYSIS

Summary of Work (5000 Words)
For the Award of Ph. D. (Sc.) Degree in Chemistry
Submitted to
The Presidency University

PRESIDENCY UNIVERSITY
KOLKATA

By
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Registration No.: R-17RS205120098
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Chapter 1: Introduction

1.1. Bioactive compound

In scientific sense, "bioactive compounds" refers to a variety of compounds that have biological activity. Bioactive chemicals in plants are defined as secondary plant metabolites that cause pharmacological or toxicological effects on humans and animals health. Bioactive substances are phytochemicals found in plants and foods to varying degrees that have the ability to change metabolic processes for the betterment of one's health [1].

1.2. Types of bioactive compounds

Figure 1 describes the four major classes of bioactive chemicals [2].

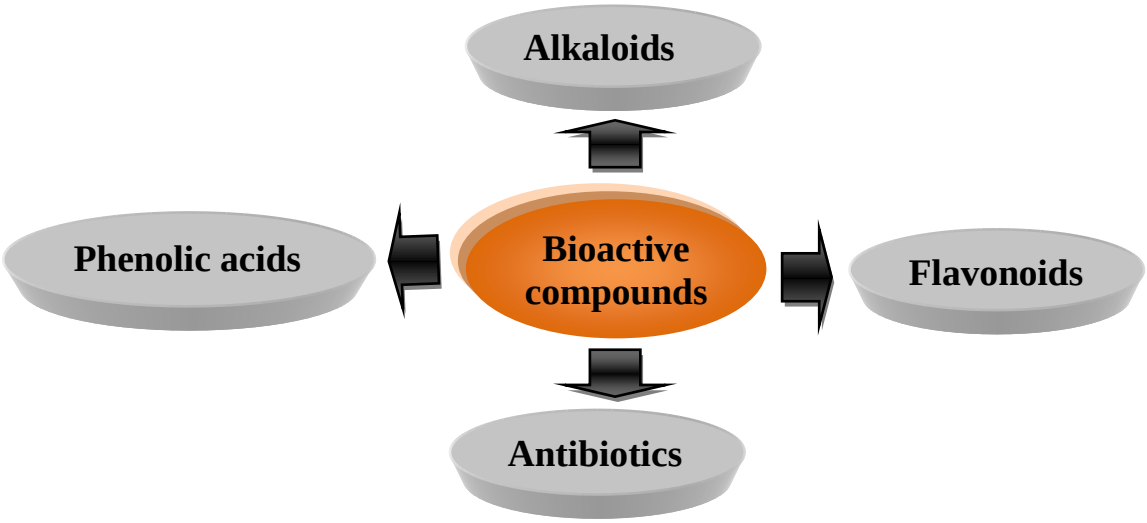


Figure 1. Types of bioactive compound

1.3. Phenolic acids (PAs)

Phenolic acids are secondary metabolites found throughout the plant world, particularly in vegetables, fruits, and a variety of beverages such as tea and coffee etc. and are utilized to combat oxidative damage illnesses such as coronary heart disease and cancer [3]. The importance of their antioxidant properties and prospective use in processed foods as natural antioxidant compounds has risen to new heights in recent years. Phenolic acid molecules and functions have been the focus of numerous agronomic, biological, chemical, and medical studies

(Fig. 2). Naturally occurring PAs can be distributed into two groups (Fig. 3): hydroxybenzoic acids (Fig. 4) and hydroxycinnamic acids (Fig. 5). Hydroxycinnamic acid molecules are synthesized as simple esters with glucose or hydroxy carboxylic acids. Plant phenolic compounds have a varied molecular structure and are distinguished by hydroxylated aromatic rings. In addition, PAs can form glycosides or esters with other natural chemicals including sterols, alcohols, glucosides, and hydroxyfatty acids in food plants. In nature, the simplest PAs are benzoic acids and cinnamic acids, which have seven and nine carbon atoms in their structural shape, respectively.

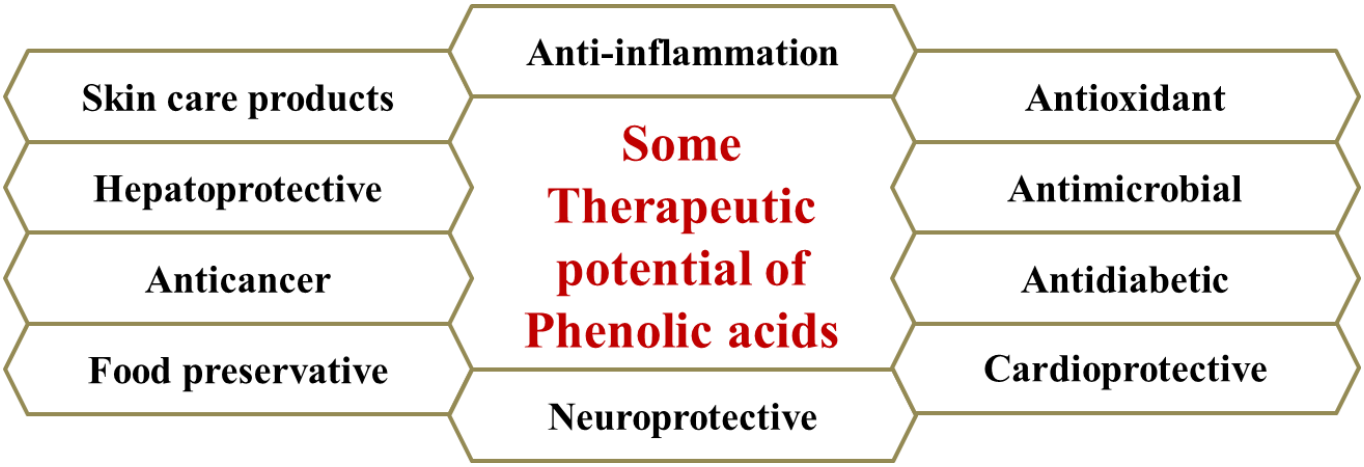


Figure 2. Some therapeutic potential of PAs.

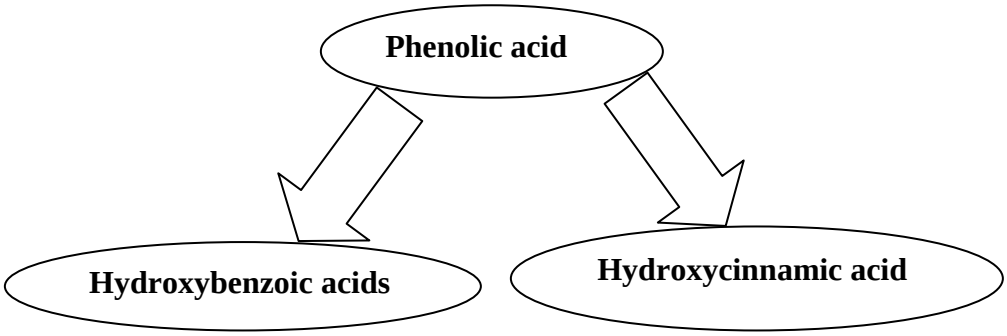
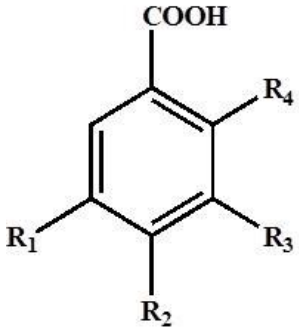
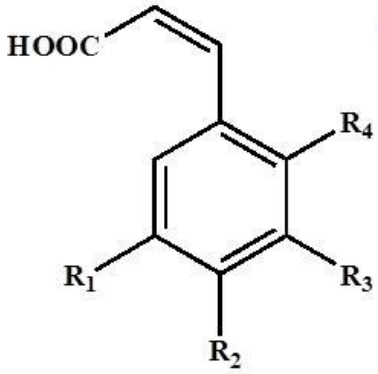


Figure 3. Classification of phenolic acids.



R_1	R_2	R_3	R_4	Common name
H	H	H	OH	Salicylic acid
OH	OH	OH	H	Gallic acid
OCH_3	OH	H	H	Vanillic acid
OCH_3	OH	OCH_3	H	Syringic acid
OH	OH	H	H	Protocatechuic acid
H	H	OH	OH	Gentisic acid

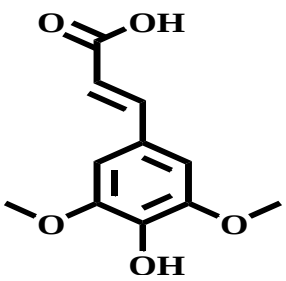
Figure 4. Some common hydroxybenzoic acids occurring in foods.



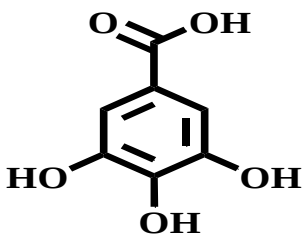
R_1	R_2	R_3	R_4	Common name
H	H	H	OH	o-Coumaric acid
H	H	OH	H	m-Coumaric acid
H	OH	H	H	p-Coumaric acid
OCH_3	OH	H	H	Ferulic acid
OCH_3	OH	OCH_3	H	Sinapic acid
OH	OH	H	H	Caffeic acid

Figure 5. Some common hydroxycinnamic acids in foods.

Due to the variety of therapeutic application of PAs', we have made an attempt to investigate the interaction and therapeutic action of some biologically significant PAs' such as (i) sinapic acid (a member of the hydroxycinnamic acid group; **Structure 1** and (ii) gallic acid (a member of the hydroxybenzoic acid group; **Structure 2** with a biological macromolecules, calf thymus DNA. Finally, we have conducted biological tests to determine the impact of alterations on the parent PAs' bio-efficacy.



Structure 1. Sinapic acid



Structure 2. Gallic acid

Chapter 2: Materials and Methods

2.1. Chemical Reagents and Equipment

The components and compounds used throughout the experiments and the production of various stock solutions are listed in Table 1. The chemicals utilised in this investigation were all analytical grade, and they were used without additional purification. Throughout the testing, double deionized water was prepared and for biological assay we used Milli-Q water. Before usage, all glassware were properly cleaned with a weak HNO_3 solution, followed by double distilled water and acetone to remove any contaminants.

Table 1. List of chemicals, including formulas and company names.

Name of the chemicals	Chemical formula	Company name
Sinapic acid	$C_{11}H_{12}O_5$	Sigma-Aldrich
Gallic acid	$C_7H_6O_5$	Loba Chemie
Calf thymus DNA (Ct-DNA)	---------	SRL
Ethidium bromide (EtBr)	$C_{21}H_{20}BrN_3$	SRL
Potassium Iodide	KI	Merck Millipore
Tris-buffer	$C_4H_{11}NO_3$	Merck Millipore
Ethyl alcohol	C_2H_5OH	Merck Millipore
MTT	$C_{18}H_{16}N_5S$	SRL
LDH-cell cytotoxicity assay kit	---------	HIMEDIA
DMSO	C_2H_6OS	Merck Millipore
PEG-4000	---------	Merck Millipore
Bromophenol blue	$C_{19}H_{10}Br_4O_5S$	Sigma-Aldrich
Nickel sulphate hexahydrate	$NiSO_4.6H_20$	LOBA CHEMIE
Beta Casein		Sigma-Aldrich

2.2. Methods:

2.2.1. UV-Vis methods

UV-vis spectroscopic measurement was performed on a Hitachi U-2910 UV-spectrophotometer at 298K using a cuvette of 1.0 cm x 1.0 cm path length.

2.2.1.1. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay

The effect of SA on radiation induced cellular toxicity after UVB radiation was determined with comparison to the cytotoxicity of control untreated cells, assigned here as 100%. Control was prepared by addition of MTT in cell free PBS. Controls were prepared in same fashion without the exposure of SA and UVB. The absorbance was measured at 590 nm.

Cytotoxicity was determined by corrected absorbance: % cytotoxicity = (100x (control-sample)

2.2.1.2. Lactate dehydrogenase (LDH) aassay

Cytoplasmic leakage of LDH in extracellular space and measurement of this LDH is an indirect way to access the extent of cellular necrosis. The absorbance was taken at 520 nm [4].

% Cytotoxicity = 100x (Corrected reading from test well - Corrected reading from untreated well)/(Corrected reading from untreated well- corrected maximum LDH release negative control).

2.2.1.3. Sialic acid assay

Sialic Acid Assay for evaluation of cell death ($O.D_{630nm}$) of human PBMC exposed to UVB radiation after prior administration of SA [5].

2.2.1.4. Radical scavenging assay

Free radical scavenging activity of SA has been studied by DPPH and ABTS assay. The absorbance were taken at 517 nm for DPPH assay and for ABTS assay absorbance were taken at 734 nm [6].

$$\text{Radical Scavenging activity (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (1)$$

2.2.2. Fluorescence

Steady-state fluorescence measurement was recorded on a Cary Eclipse fluorescence spectrophotometer using 1.0 cm quartz cells.

2.2.3. Molecular Docking

The molecular docking approach may be used to represent the atomic level interaction between a small molecule and DNA [7]. The docking procedure consists of two main steps: predicting the ligand structure as well as its position and orientation within these sites (known as pose) and determining the binding affinity.

2.2.4. Circular dichroism (CD) spectroscopy

It is mostly used to study biological molecules, their structure, and interactions with metals and other molecules [9, 8].

2.2.5. Fourier Transform Infrared Spectroscopy (FTIR)

Using a hydraulic press (5 tonne pressure), a tiny portion of the solid sample was completely combined with dried KBr to form a pellet. NPs treated with metal salt solution were also extracted and dried for FTIR spectral analysis and confirmation of chemical interactions.

2.2.6. Transmission electron microscopy (TEM)

5 mg of solid material were dispersed in 0.5 mL of water solvent and sonnicated for 30 min. On a carbon-coated Cu grid, a drop of this solution was dropped. After drying the grid under an IR lamp, the TEM microscopic pictures were acquired.

2.2.7. Powder X-ray diffraction (PXRD)

The powdered Ni-SA nanoparticle (NPs) samples were taken in a rectangular metal holder and lightly pressed for the X-ray diffraction studies.

2.2.8. Isothermal Titration Calorimetry (ITC)

This approach was used to investigate the interaction of NPs with the $KMnO_4$ ion. This study may be used to determine thermodynamic parameters such as change in enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG) and other parameters such as the association constant (K_a) and stoichiometry for the interaction between two or more compounds with the help of following equation (Eq. 2) [10].

$$\Delta G = \Delta H - T\Delta S \qquad (2)$$

The value of ΔG can also be obtained from the value of association constant (K_a) with the help of following equation (Eq.3)

$$\Delta G = -RT \ln K_a \qquad (3)$$

Where, R is the universal gas constant.

Chapter 3A: Interaction of Sinapic acid with calf thymus DNA.

Sinapic acid (SA) is commonly present in human diet. Due to its wide variety of pharmacological activities it has attracted much attention for the researchers. In this work we have demonstrated a detailed investigation of the binding interaction between sinapic acid with Ct- DNA.

3.1. UV-vis absorption spectroscopy

SA exhibited two absorption peaks at ~226 nm and ~306 nm for π-π^* and n- π^* transitions respectively, whereas, Ct-DNA displayed maximum absorption at ~260 nm (Fig. 6) [12]. Fig. 6A displayed a hypochromic effect without any significant shift at ~306 nm and ~226 nm. This observation suggested a weak interaction between SA and Ct-DNA.

The double reciprocal plot of $1/(A_0 - A)$ versus 1/[DNA] at 298 K is shown in Fig. 6B. The calculated binding constant is 4.68 x 10^3 M^{-1}. The lower K value indicated a weak interaction between SA and Ct-DNA.

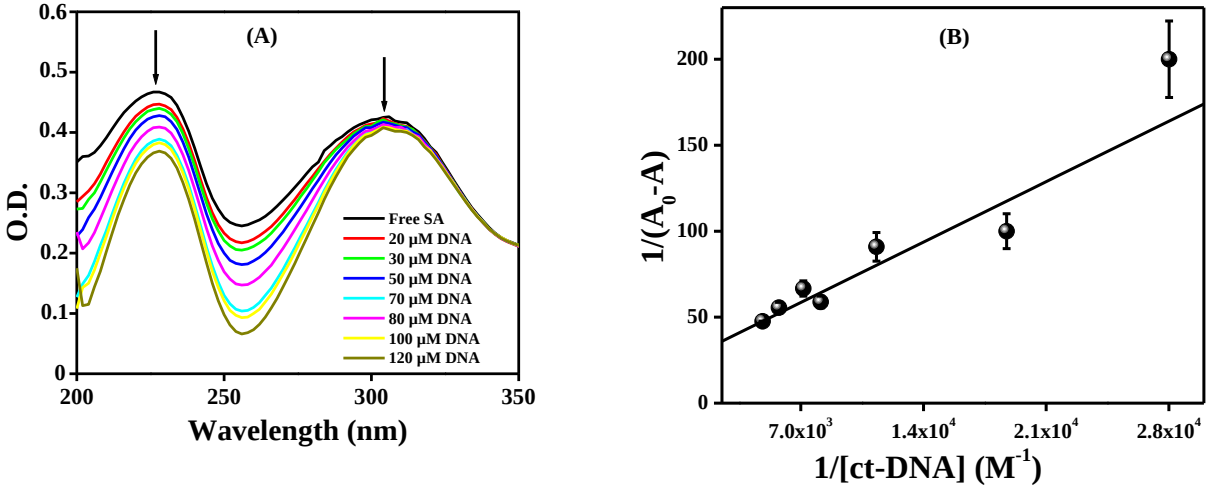


Fig. 6. The absorption spectra of SA and Ct-DNA (A). The double reciprocal plot (B).

3.2. Steady-state fluorescence spectroscopy

The quenching of intrinsic fluorescence intensity of SA (~430 nm) in the presence of ct-

DNA indicates a complex formation (Fig. 7A). To ascertain the quenching mechanism, the following Stern-Volmer equation was employed (equation 4).

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + k_q\langle\tau_0\rangle[Q] \qquad (4)$$

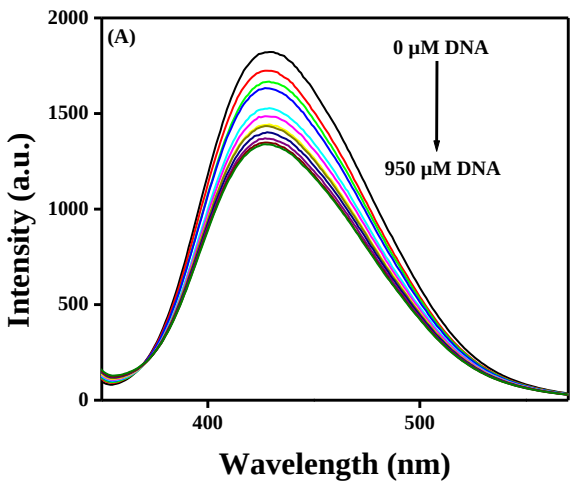
A steady increase of Stern-Volmer quenching constant (K_{SV}) values (Fig. 7B; Table 2) with increasing temperature convincingly suggested the presence of dynamic quenching mechanism. The bimolecular quenching constant (k_q) values (Table 2) are somewhat higher than the maximum threshold collision quenching constant ($2x10^{10}$ $M^{-1}s^{-1}$) which supports the occurrence of static quenching process [13]. Again, the activation energy as calculated from Arrhenius equation (Fig. 7B inset; equation 5), was found to be 14.83 kJ mol^{-1}, which is somewhat higher as compared to the other biomolecular reactions, clearly indicated the presence of static quenching process. Also, the modified Stern-Volmer quenching constant (K_a) (equation 6) vary appreciably with temperature which supports the static quenching mechanism.

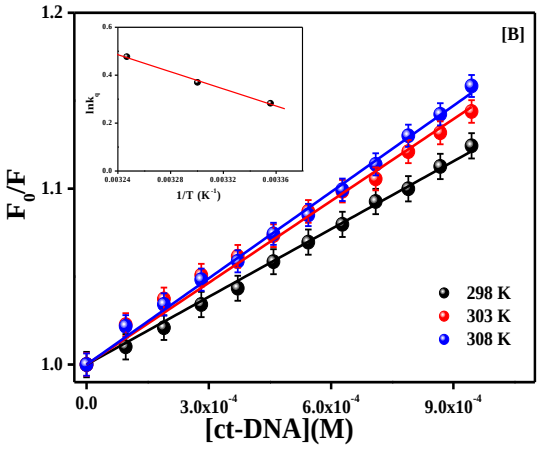
$$\ln k_q = \ln(K_{sv}/\langle\tau_0\rangle) = -E_a/RT + \ln A \qquad (7)$$

$$\frac{F_0}{\Delta F} = \frac{F_0}{(F_0 - F)} = \frac{1}{f_a K_a}\frac{1}{[Q]} + \frac{1}{f_a} \qquad (6)$$

A gradual decrease in binding constant value (K_b) with increasing temperature pointed towards the destabilization of the complex at a higher temperature (equation 7; Table 3; Fig. 8A).

$$\log\frac{(F_0 - F)}{F} = \log K_b + n\log[Q] \qquad (7)$$





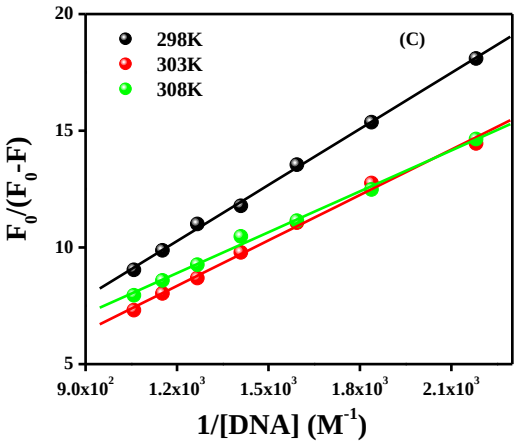


Fig. 7. Fluorescence quenching spectra (A) and Stern-Volmer plot (B). Inset: Arrhenius plot. Modified Stern-Volmer plot (C).

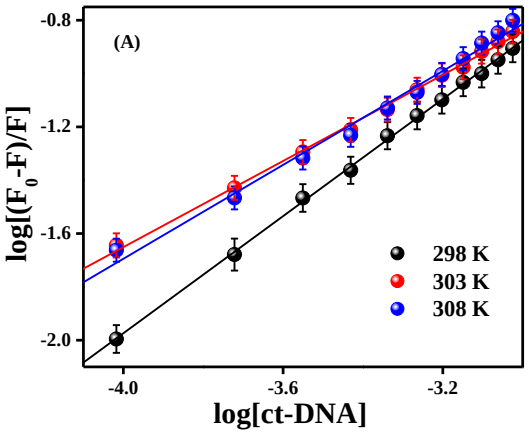
3.3. Evaluation of binding constant and thermodynamic parameters

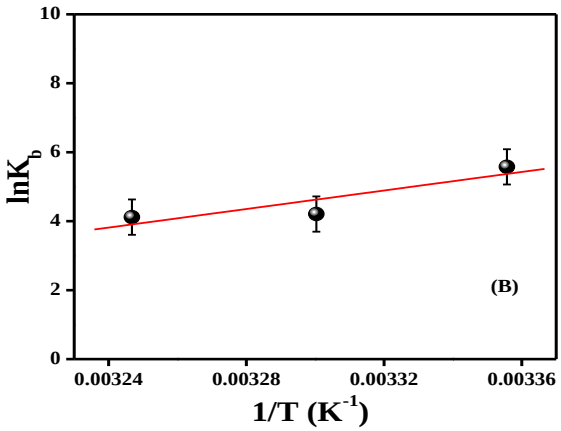
Thermodynamic parameters like ΔG, ΔS and ΔH were analyzed using the following van't Hoff equation (equation 8; Fig. 8B and 8C; Table 3) [13]: According to Ross and Subramanian [14] the interactive forces can be classified as follows based on the values of ΔS and ΔH:

$$\ln K_b = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (8)$$

$$\Delta G = \Delta H - T\Delta S \qquad (9)$$

Both ΔH and ΔS are negative, corresponding to the presence of van der Waals interaction and hydrogen bond formation during complex formation of SA with Ct-DNA. The negative sign for ΔG indicated the spontaneity of the binding process.





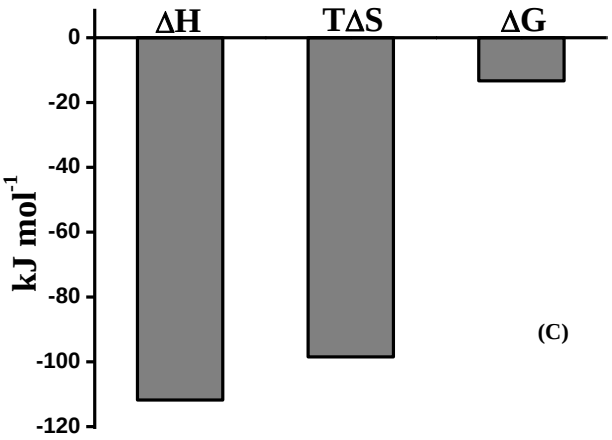


Fig. 8. The binding constant plot (A), van't Hoff plot (B) and the thermodynamic parameters plot at 298 K (C).

Table 2. $\mathbf{K_{SV}}$ and $\mathbf{k_q}$ and modified Stern-Volmer quenching constant at three different temperatures for SA-Ct-DNA system.

T (K)	K_{SV} (M^{-1})	R^2	k_q ($M^{-1}s^{-1}$)	K_a (M^{-1})
298	132.69	0.998	$1.327x\ 10^{11}$	$0.823x10^2$
303	144.83	0.996	$1.448x10^{11}$	$0.870x10^2$
308	161.21	0.996	$1.612x10^{11}$	$3.23x10^2$

Table 3. Binding constant and other thermodynamic parameters at three different temperatures.

T (K)	K_b (M^{-1})	n	R^2	ΔH (kJ mol^{-1})	ΔS (J mol^{-1})	ΔG (kJ mol^{-1})
298	263.8	1.09	0.998	-111.78	-330.46	-13.30
303	67.18	0.88	0.992			-11.65
308	37.92	0.81	0.996			-10.00

3.4. Competitive displacement assay

The fluorescence spectra of the ethidium bromide-CtDNA (EB-CtDNA) complex in the absence and presence of SA is shown in Fig. 9. Thus these results prove that SA binds to Ct-DNA via non-intercalative binding mode.

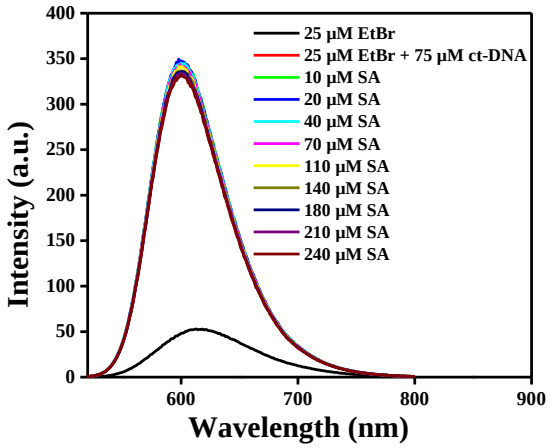


Fig. 9. Fluorescence emission spectra of native ethidium bromide and ethidium bromide-CtDNA complex with increasing concentration of SA.

3.5. Iodide quenching study

Table 4 (Fig. 10) shows that in the absence and presence of Ct-DNA, the values of K_{SV} was found to be 6.36 M^{-1} and 6.39 M^{-1} respectively. The lesser degree of reduction in the K_{SV} values indicated groove binding mode.

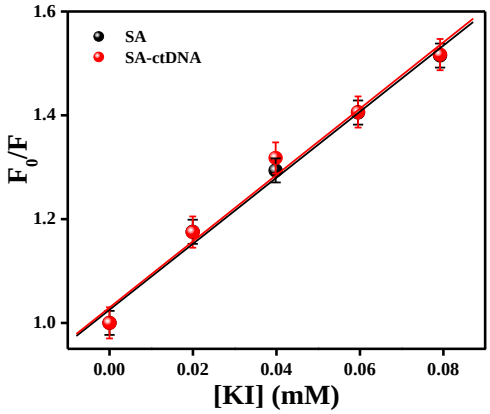


Fig. 10. Stern-Volmer plot.

Table 4. K_{SV} values obtained in KI quenching of SA in the absence and presence of Ct-DNA.

System	K_{SV} (M^{-1})	R^2	Change in K_{SV} (%)
SA	6.36	0.9949	-
SA + Ct-DNA	6.39	0.9917	0.47

3.6. DNA melting experiment studies

The calculated melting temperature values of Ct-DNA and SA-CtDNA complex appeared at 66.2 °C and 67.4 °C respectively (Fig 11), obtained from the curves in Fig. 11. The small change in the T_m suggested groove binding.

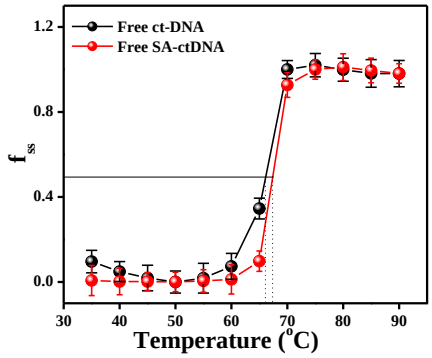


Fig. 11. DNA melting plot.

3.7. Circular dichroism (CD) studies

The CD profile confirms the binding mode of the fluorophore SA to the host DNA to occur through groove binding [8].

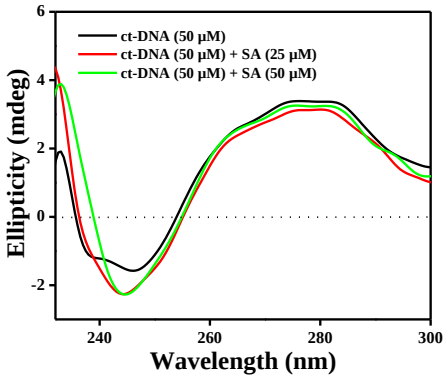


Fig. 12. Far-UV CD spectra.

3.8. Molecular docking

Sinapate was found to bind exclusively in the minor groove of DNA and specifically in the GC rich region with binding energy -24.14 kJ/mol (25.52 kJ/mol in AutoDock Vina) (Fig. 13). It can also form a hydrogen bonding interaction through its methoxy group with the $-NH_2$ group of guanine base (Fig. 14A; 14B; 14C).

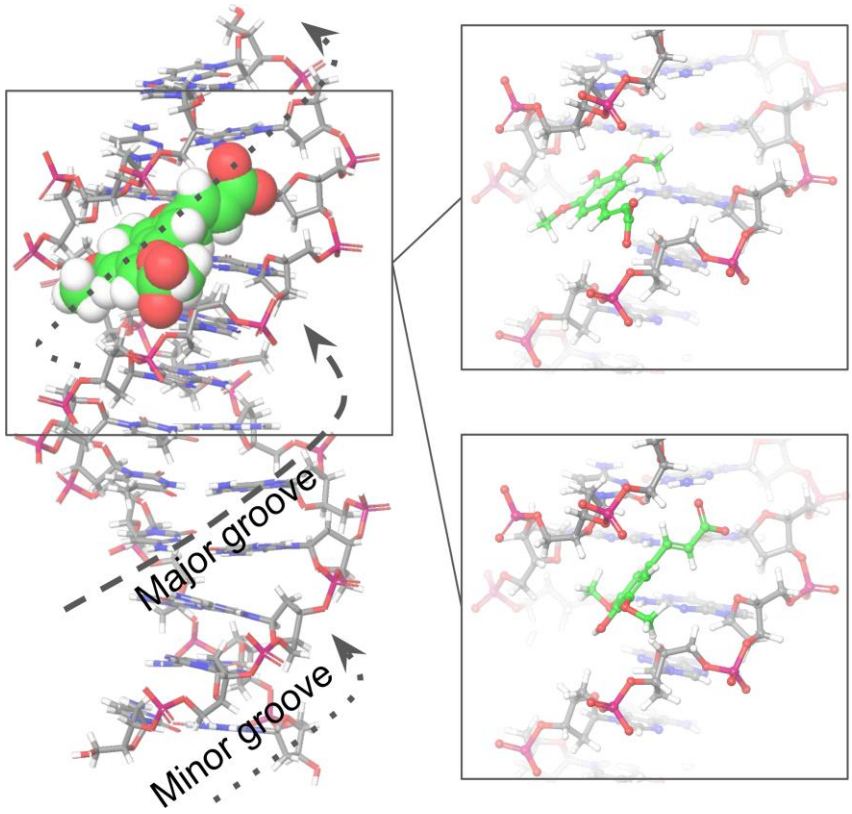


Fig. 13. Binding of sinapate (green) in the minor groove of DNA.

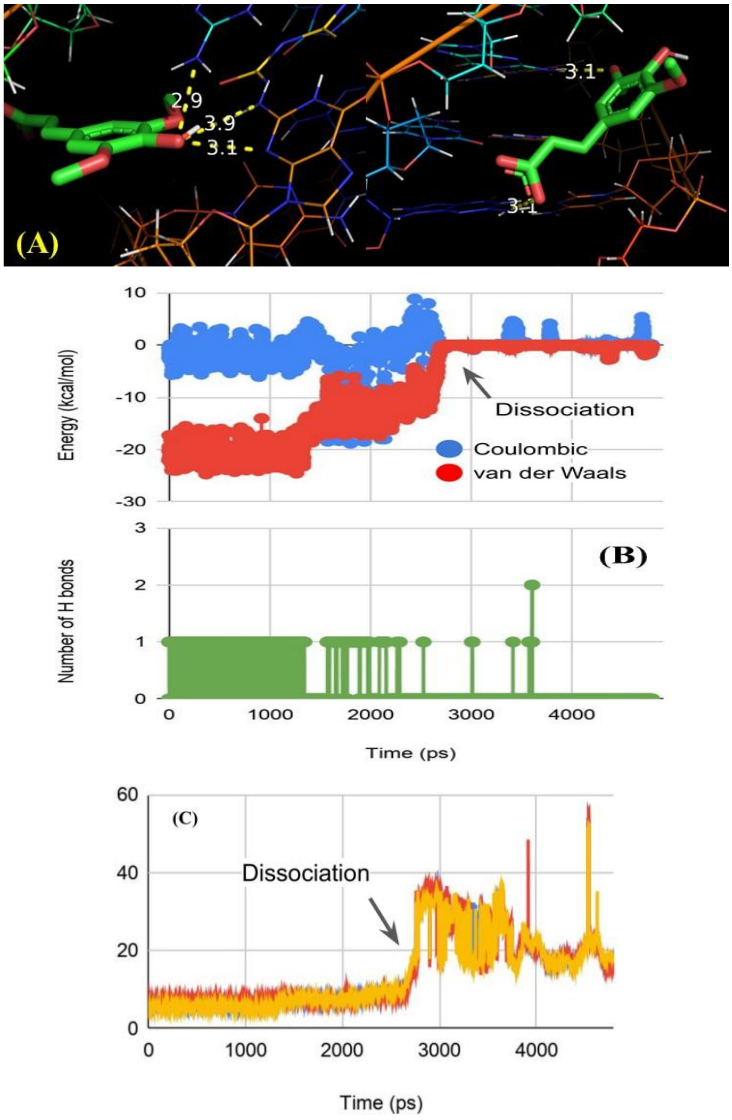


Fig. 14. (A): Hydrogen bonds between SA and the bases of DNA. (**B):** Binding energy of SA with DNA and the H bonding over the period of simulation. **(C):** Distance between C1 of SA and the -NH2 group of three adjacent three guanine bases viz. G10 of chain A (blue) G14 of chain B (red) and G16 of Chain B (yellow). Point of dissociation of SA from DNA is marked.

Chapter 3B: In vitro application of Sinapic acid

Ultraviolet radiation (UVB) is a potent environmental carcinogen; it induces oxidative and inflammatory skin damage and eventually cancer. Naturally occurring polyphenols are potent antioxidants and anti-inflammatory agents which can potentially reduce the damaging effects of UVB rays on the human body. To evaluate the biological activity and efficacy of candidate solvent for transporting SA into the blood and its bioavailability at the blood and target site we have done *in vitro* study on human blood peripheral mononuclear cells (PBMC). Radiation-induced damage of blood cells has been studied by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay for cell viability and by Lactate dehydrogenase (LDH) cytotoxicity assay and sialic acid assay with and without SA administration in the blood *in vitro* prior to UVB exposure.

4.1. MTT assay:

The %viable cell in case of alcohol/buffer system was higher than in DMSO/buffer and SA dissolved in PEG media. The observation primarily indicated that even a concentration of 10 µM of SA was able to prevent cell damage remarkably against UVB radiation (Figure 15).

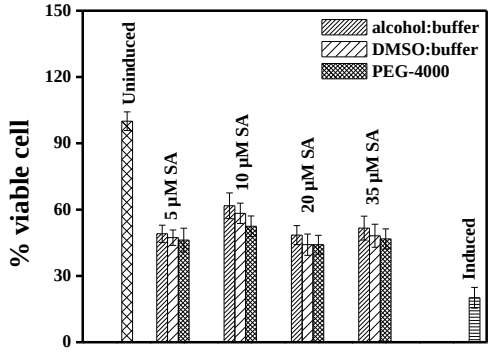


Fig. 15. Comparative analysis of percent viable cells (analyzed by MTT assay).

4.2. LDH assay:

The observation primarily indicated that a concentration of 35 µM of SA was able to prevent cell damage mostly against UVB radiation (Fig. 16).

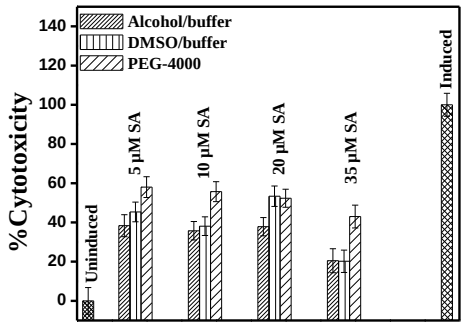


Fig. 16. LDH Assay for % cytotoxicity.

4.3. Sialic acid assay

The absorbance value was lowest at a 10 µM concentration of SA, which suggested a lesser release of sialic acid from irradiated blood cell at that particular concentration (Fig. 17).

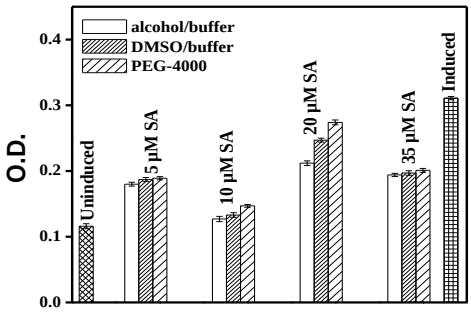


Fig. 17. Sialic Acid Assay for evaluation of cell death ($O.D_{630nm}$).

4.4. DPPH and ABTS assay:

Free radical scavenging activity of SA has been studied by DPPH and ABTS assay. It is seen from the result of DPPH activity that gradual increase in SA concentration increases the free radical scavenging activity of SA in a dose-dependent manner. A similar result has also been observed when the ABTS assay performed to evaluate the Trolox equivalent (Fig. 18).

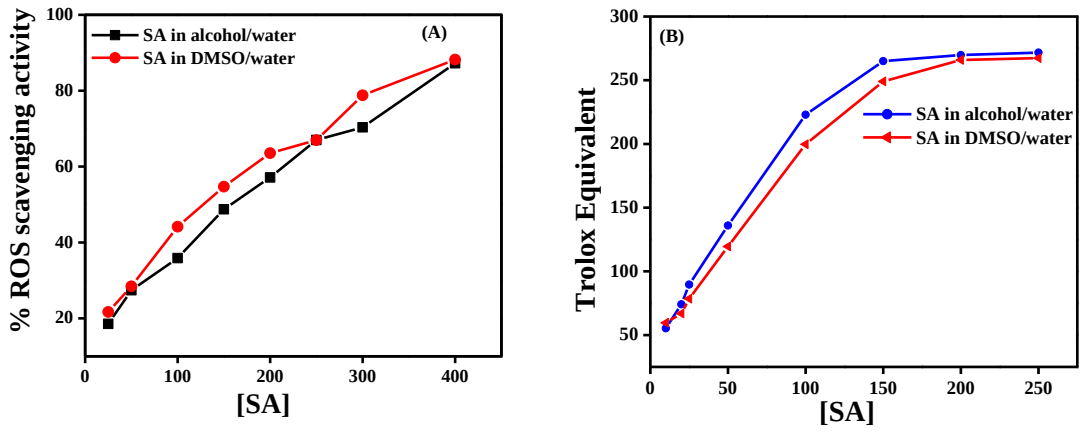


Fig. 18. (A) ROS scavenging activity of SA by DPPH activity. (B) ROS scavenging activity of SA measured by Trolox equivalent through ABTS activity.

Chapter 4: Ni-Sinapic Acid Nanocomposite in the Selective Sensing of Permanganate ions

In recent years, metals like Fe, Co and Ni have been used to synthesize nanoparticles for their beneficial properties and applications in different fields. Ni-based nanoparticle can be a good candidate for various pharmaceutical syntheses. In this work, we have synthesized a Sinapic acid based Ni nano-particle to see any possible sensing effect of this nano-particle.

5.1. Synthesis of Sinapic acid (SA) based Ni nanoparticle (Ni-NPs)

At first we prepared 10 mM SA solution in 1:4 water-alcohol medium at pH~11. Then in another beaker we prepared 10 mM $NiSO_4$ solution in distilled water. Equal amount of these two solutions were added and kept in 45 °C temperature for 24 hours. After that the solution part was evaporated using IR-lamp. The residue was washed with 1:4 water-alcohol system and collected for further experiments.

5.2. Visual observation

Initially a visual colour change was observed for the synthesis of Ni-NPs (Fig. 19). Upon addition of $NiSO_4$ solution to the SA solution, the colour of the sinapic acid solution changed from light yellow to brown indicating the formation of nanoparticles. The Ni-NPs were further characterized using different techniques.

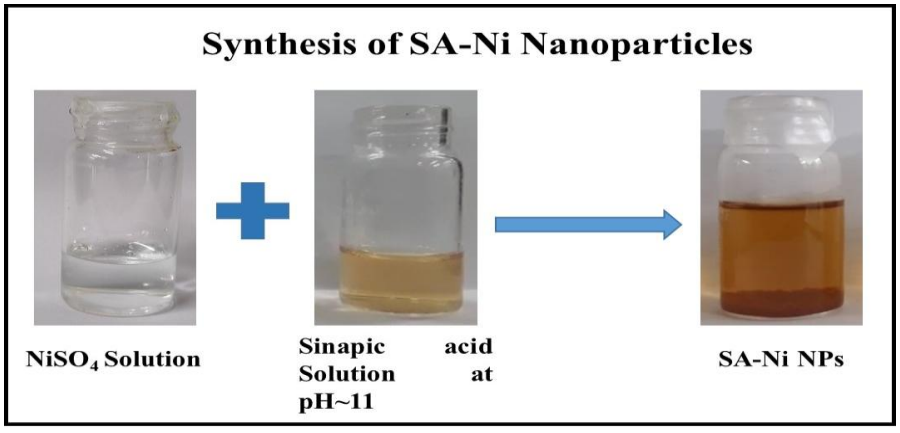


Fig.19. Photograph of synthesis of SA-Ni NPs.

5.3. Fourier Transform Infrared Spectroscopy (FTIR)

The region (a) around 3500-2400 cm^{-1} was observed mainly due to the hydroxyl group and -CH_3 group, region (b) around 1800-1000 cm^{-1} was observed for C=O stretching frequency of –COOH group, aromatic ring, and C-O-C stretching frequency, and region (c) around 1000-500 cm^{-1} was formed mainly due to the bending vibrations. The result showed that the admissible change in the vibrational frequency was observed mainly in region (a) and region (b) suggesting the participation of the –COOH group along with the benzene ring of the SA towards the complexation with $NiSO_4$.

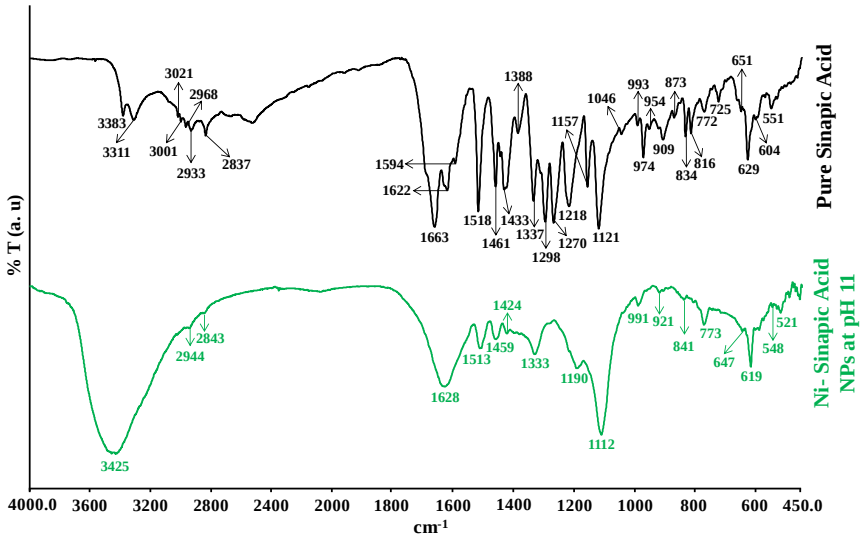


Fig. 20. FTIR spectra of pure sinapic acid and NPs.

5.4. Transmission electron microscopy (TEM)

From TEM studies it was evident that the particle size was around 5 to 10 nm. Clear lattice fringes were observed from the TEM image suggesting the nano crystallinity of the material (Fig. 21A). The selected area electron diffraction (SAED) pattern of the material showed some concentric rings, again confirming the nano crystallinity of the material (Fig. 21B).

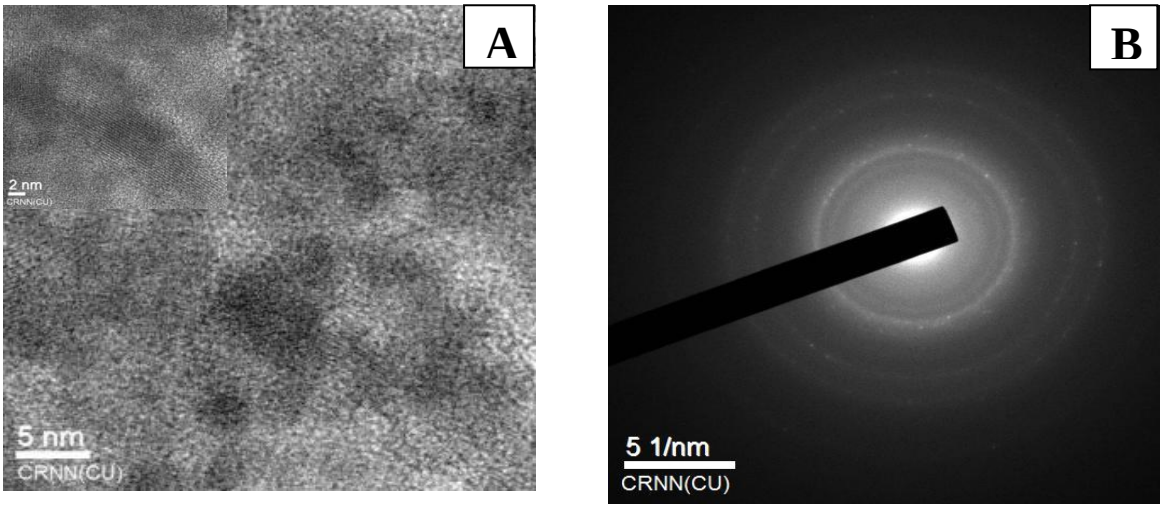


Fig. 21. TEM image of the material and inset for closer view (A) and SAED pattern of the material (B).

5.6. Sensing of permanganate ion

There was no spectral change observed for any of the analytes except permanganates ions. Gradual addition of permanganate ions resulted in formation and decrease of characteristic absorption peak of the Ni-NPs (300 nm) (Fig. 22). The new peak at 603 nm represents the interaction between Ni-NPs and the permanganate ion and increase in absorption up to 2 µM concentration of the analyte. The limit of detection is 413 nM and the linear range for this detection is 0.5 to 1.7 µM with correlation coeffitient of 0.947.

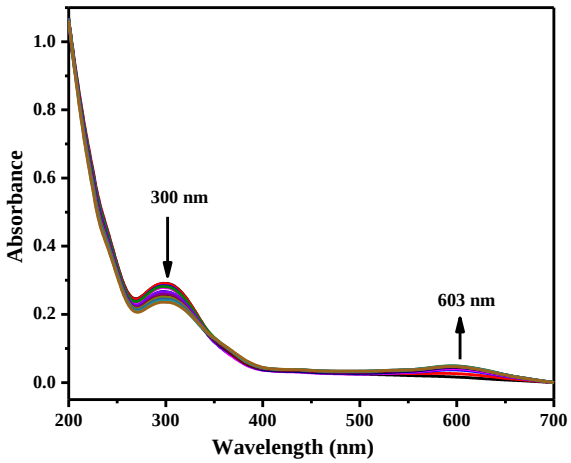


Fig. 22. Spectral change of NPs solution in presence of permanganate ion (0-3 µM)

5.7. Isothermal titration calorimetry (ITC)

ITC measures the heat changes upon the binding of two or more molecules in solution. ITC can provide the association constant (K_a), reaction stoichiometry (n), the heat capacity (ΔCp) of the reaction, binding free energy (ΔG), entropy (ΔS), and enthalpy (ΔH). The interaction between $KMnO_4$ with the Ni-NPs was found to be 1:1, with K_a of 15.43 x 10^3 M^{-1} (Fig. 23). The ΔH and ΔS changes associated with this interaction are -80000 cal/mol and -2972 cal/mol/deg, respectively. The ΔG value obtained from the experiment is -5720 cal/mol. The interaction process between $KMnO_4$ with the Ni-NPs appears to be an enthalpy driven process.

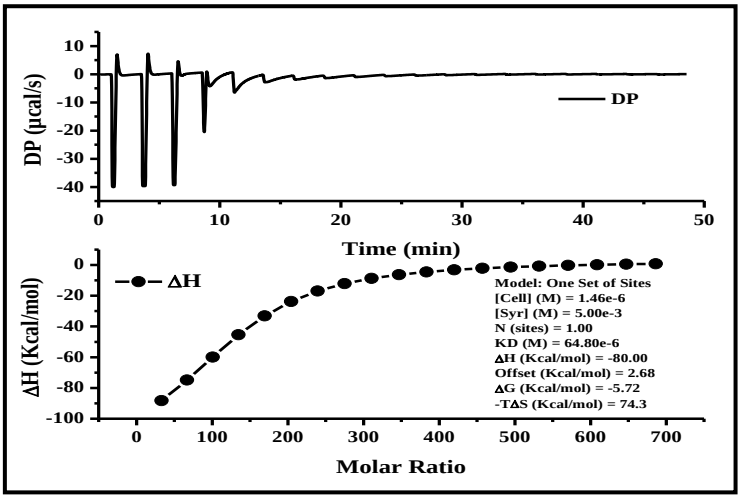


Fig. 23. ITC data for the binding isotherm between KMnO4 and the NPs, At 298 K.

5.8. Interference study

The interference experiment was carried out in the presence of other cations and anions at the same concentration as that of MnO_4^- ion. The obtained results indicated that there was no significant interference from any of the ions in the detection of trace amounts of MnO_4^- ion (Fig. 24).

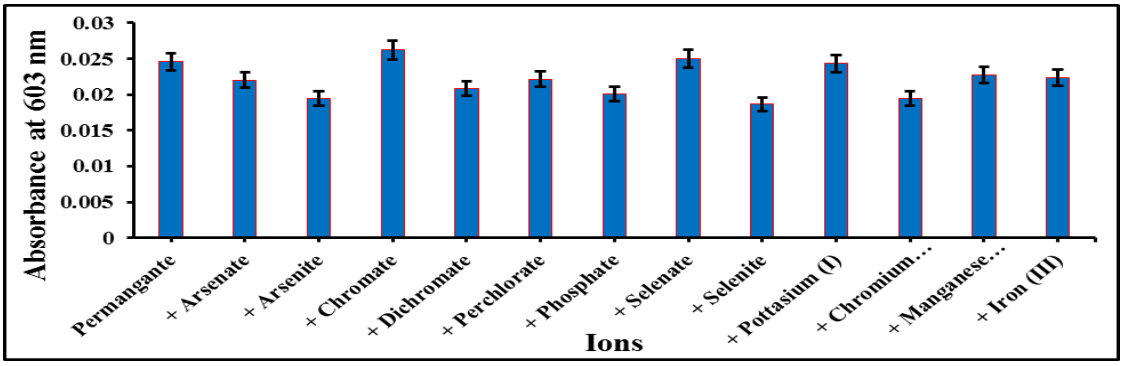


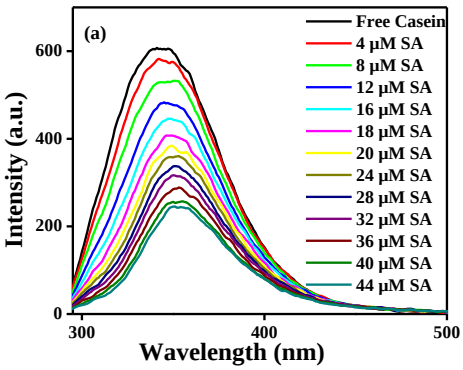
Fig. 24. Bar plot of other interference ions.

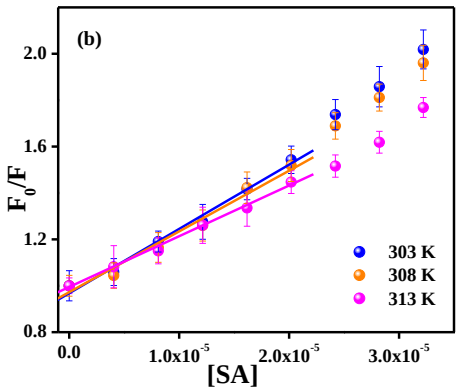
Chapter 5: Sinapic acid encapsulation in β-casein micelles

Food proteins are of particular interest due to their high emulsification, gelation, and water binding and foaming ability. Bovine β-Casein (β-CN) is one of the milk protein and used as an oral drug delivery system [15, 16]. SA is a well-known drug for enormous biological activities such as anti-inflammatory, anti-oxidant, anti-bacterial, anti-cancer, anti-anxiety [7]. The present study aims to achieve detailed quantitative information on the characteristics of the drug-loaded micelles, in the bulk solution, emphasizing the investigation of drug conformation, packing properties, and aggregation state.

6.1. Fluorescence Studies

With the addition of SA, the fluorescence intensity of β-CN (350 nm) gradually decreased, demonstrating the interaction with SA (Fig. 25a). With increasing temperature, the K_{SV} values (Fig. 25b and Table 5) declined, and the k_q values were in the order of 10^{13}. As a result, static quenching drove the interaction between β-CN and SA. The k_b values were in the range of 10^5 M^{-1}, showing that SA and β-CN bind moderately (Fig. 25c). Furthermore, both ΔH and ΔS are negative (Fig. 25e and Table 5), implying that van der Waals interaction, hydrogen bond formation are involved in the interaction of β-CN with Sinapic acid.





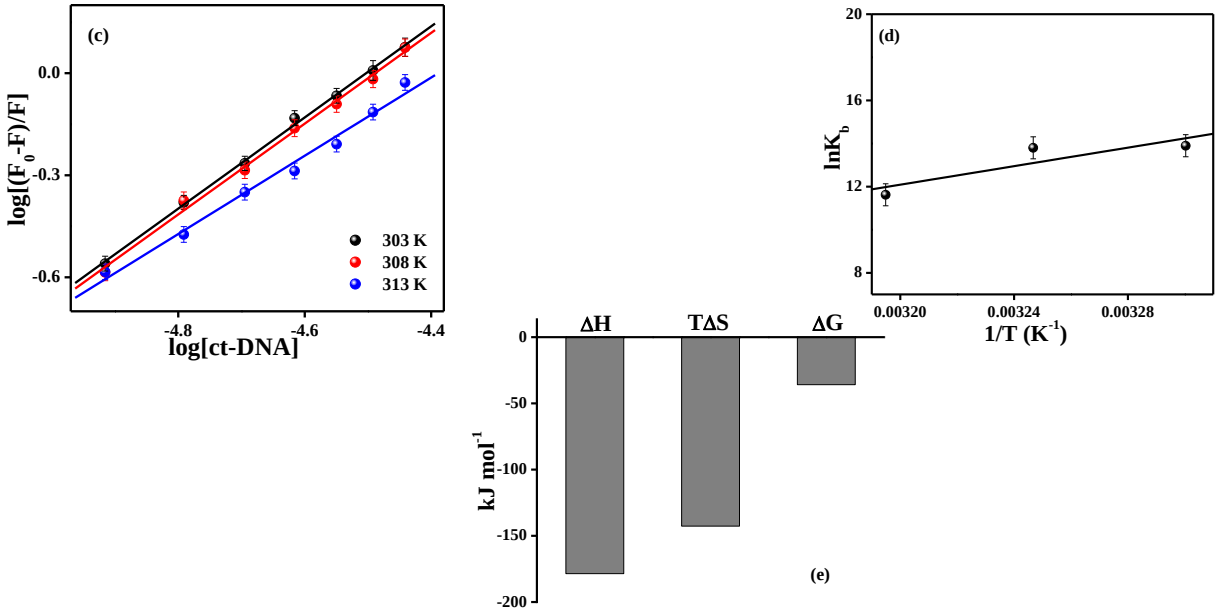


Figure 25. (a) Fluorescence titration of βCN (5 μM) with sinapic acid at 298 K; The corresponding (b) Stern-Volmer, (c) binding, (d) van't Hoff plot for three different temperatures; and (e) Thermodynamic plot; λex = 280 nm.

Table 5. List of Stern-Volmer, binding, and thermodynamic parameters

System	Temp. (K)	K_{SV} (M^{-1})	k_q ($M^{-1}s^{-1}$)	k_b (M^{-1})	n	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (kJ mol^{-1})
SA- βCN	303	2.77×10^4	2.77×10^{13}	11.48×10^5	1.34	-178.56	-470.87	-35.89
	308	2.61×10^4	2.61×10^{13}	9.77×10^5	1.33			-33.53
	313	2.18×10^4	2.18×10^{13}	1.09×10^5	1.14			-31.18

Chapter 6: Interaction of Gallic acid with Ct-DNA.

The present study shows a detailed characterization of the binding mode of a potential antioxidant and anti-carcinogenic fluorescent drug, gallic acid (3,4,5-trihydroxybenzoic acid; GA) with calf thymus DNA (Ct-DNA).

7.1. Iodide quenching study

The K_{SV} value of the DNA bound GA was almost similar to that of free GA indicating that the mode of binding of GA to Ct-DNA is groove (Fig. 27) [13].

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \qquad (8)$$

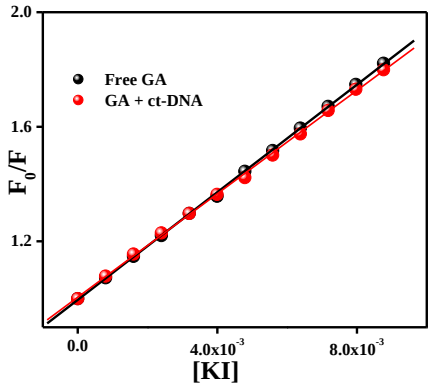


Fig. 27. Stern-Volmer quenching plot.

7.2. CD experiment

Both the bands of the CD spectrum of Ct-DNA does not changed significantly, indicating groove binding mode (Fig. 28).

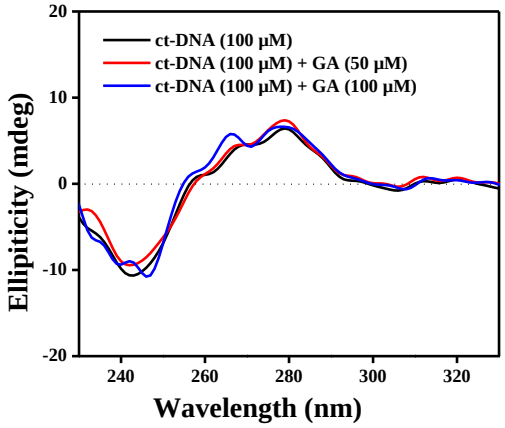


Fig. 28. Far-UV CD spectra.

7.3. Competitive binding experiment

In our study, GA could not alter the fluorescence intensity of DNA-EB complex thus concluded that GA binds to Ct-DNA through non-intercalative pathway (Fig. 29).

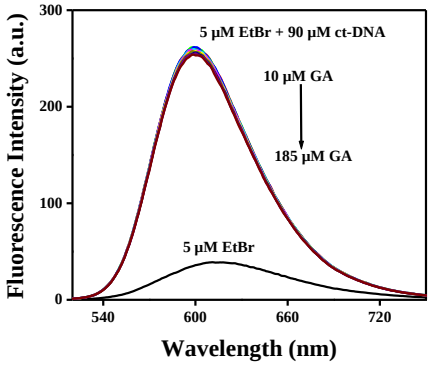


Fig. 29. Fluorescence emission spectra of competitive experiment.

7.4. DNA melting:

The values of T_m for Ct-DNA and GA-Ct-DNA complex were 62 °C and 63 °C respectively. These experiments further evidence the possibility of groove binding between Ct-DNA and GA (Fig. 30) [17].

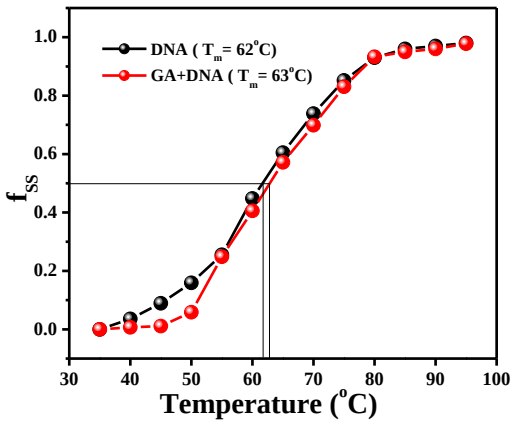


Fig. 30. Melting curve of free Ct-DNA and GA-ctDNA complex.

7.5. Molecular Docking Study:

Docking studies with DNA and Gallic acid (GA) reveled groove binding with a binding affinity value of -5.4 kcal/mol. It was found that GA interacts with DNA bases through hydrogen bonding interaction.

The docked structure showed that GA binds to the major groove of B-DNA without showing any intercalation.

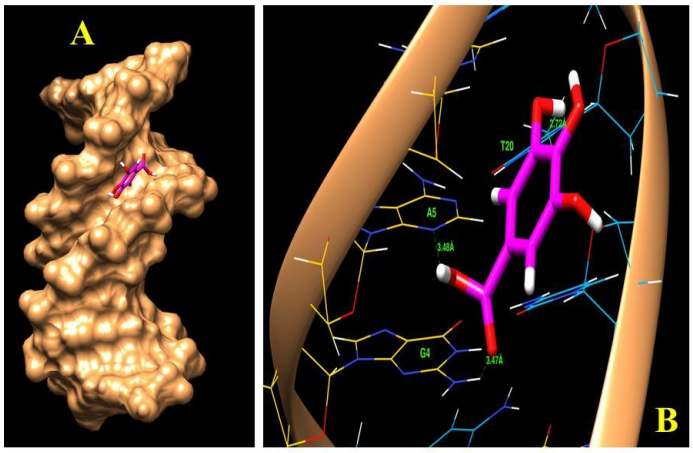


Fig. 31. Docking structure of GA and Ct-DNA

Table 6. Hydrogen bnding distance between GA and nearby DNA bases pairs.

DNA residue	Gallic acid atom	Distance (in Å)
Adenine (A5-N)	OH (of COOH)	3.48
Guanine (G4-NH_2)	C=O (of COOH)	3.47
Thymine (T20-C=O)	O-H (meta hydroxyl group)	2.72

Chapter 7: Conclusion

In In my works I have investigated SA and GA with calf thymus DNA and studied the mode of their interaction. Initially the interaction was found to be simple minor groove binding. Then the biological efficacy of SA was evaluated and the optimum concentration of SA was calculated using different assay techniques like MTT, LDH and sialic acid assay and 10 µM was found to be the best value. Interestingly, SA show good antioxidant activity in alcohol/buffer medium. SA was further investigated by preparing NPs with Ni and it was found to sense MnO_4^- ions. After that we have use a distinct technique to increase the bio-efficacy of SA by preparation of micelle entrapment in βCN. A detailed spectroscopic study revealed a favorable binding interaction between SA and βCN. Besides, the entrapment of SA in βCN system enhances the antioxidant capacity of free SA. Finally, we also investigate the detailed interaction of GA (one of the SA derivative) with Ct-DNA and the results show that the interaction is non-intercalative major grove.

Chapter 8: Limitations and future scopes

The following limitations must be highlighted in relation to these works:

- One of the selected phenolic compounds, GA, emits in the region of Ct-DNA that prevented us to decipher the interaction mechanism using fluorescence spectroscopy.
- Interaction mechanism of dietary PAs with Ct-DNA could have been enhanced further with the assistance of certain advanced instruments/methods.
- The enhanced bio efficacies of dietary PAs can be observed via both *in vivo* and *in vitro* methods. However, we could only perform few *in vitro* methods.

Some future aspects of these works:

- Further modification of the delivery systems can be done in order to achieve better results with targeted delivery.
- A better understanding of drug release (*in vivo* and *in vitro*) can be obtained by using fluorescent dietary PAs.
- The impact of incorporating these encapsulated dietary PAs into specific food matrices on their quality attributes (such as appearance, texture, stability, and flavor profile) can be determined.

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List of Publication

1. Mondal, P., and Bose, A., 2019. Spectroscopic overview of quercetin and its Cu (II) complex interaction with serum albumins. *BioImpacts: BI*, *9*(2), p.115.
2. Mondal, P., Sengupta, P., Pal, U., Saha, S. and Bose, A., 2021. Biophysical and theoretical studies of the interaction between a bioactive compound 3, 5-dimethoxy-4-hydroxycinnamic acid with calf thymus DNA. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *245*, p.118936.
3. Mondol, P., Bose, A. and Chanda, S., 2022. Evaluation of Sinapic acid to ameliorate ionizing radiation induced peripheral blood mononuclear cell death. *Research Square* (In Press, 2022).

Others

1. Sengupta, P., Pal, U., Mondal, P. and Bose, A., 2019. Multi-spectroscopic and computational evaluation on the binding of sinapic acid and its Cu (II) complex with bovine serum albumin. Food chemistry, 301, p.125254.
2. Sengupta, P., Mondal, P., Mukherjee, S., Chanda, S. and Bose, A., 2020. Rutin-serum albumin interaction in different media and its effective dose selection in radiation-induced cytotoxicity on human blood cells. Journal of Herbal Medicine, 21, p.100322.

Seminars attended

Name of the Seminar	Organized by	Year	Title of poster presentation
Two Day Symposium	Department of Chemistry, Presidency University, Kolkata.	2018	Chemical and biological interaction of a phenolic acid with Bovine Serum Albumin and Hen blood in view of spectroscopic approach.
Frontiers in Modern Biology (FIMB) 2018	Indian Institute of Science Education and Research Kolkata, Mohanpur Campus, India.	2018	Evaluating the bioavailability of Sinapic acid in three different solvent systems and its biological efficacy on UV radiation exposed cells in vitro.
4 Regional Science & Technology Congress-2019 Southern Region	Department of Science & Technology and Bio-Technology, Government of West Bengal. India.	2019	Deciphering the mode of interaction between bioactive compound Sinapic acid with calf thymus DNA in light of spectroscopic and theoretical tools.

International Seminar on "Innovation, Expansion, Impacts and Challenges in Chemical and Biological Sciences" (ICBS-2020)	Department of Chemistry, Surendranth College, Kolkata, India.	2020	Exploration of the binding interaction between a bioactive phenolic compound with Calf thymus DNA.
National Webinar on "Aspects of Material Chemistry in Modern Times"	Department of Chemistry, Presidency University, Kolkata 73, India.	2020	---
The two days webinar on "Gaussian, ChemDraw and Mnova Software"	Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore and Scube Scientific, New Delhi.	2020	---
An International Webinar on "The Emerging Pandemic Crisis: Physiological and Socio-environmental Imperatives"	Department of Physiology, Sister Nibedita Govt. General Degree College for Girls, West Bengal, India.	2020	---
A One Day International Webinar on "Recent trends in Chemistry in the fields of Medicine and Industry"	Department of Chemistry, Presidency University, Kolkata, India.	2020	---

Forwarded

Signature of the Candidate

(Dr. Adity Bose)

(Prasenjit Mondal) **Supervisor**

Assistant professor of Chemistry

Presidency University, Kolkata.

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SL. NO. Science/2017/15

PRESIDENCY UNIVERSITY
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Certificate of Enrollment

This is to certify that upon approval by the Ph.D. Committee of the Department of ***CHEMISTRY*** *and confirmation by the Faculty Doctorate Committee, and subsequently followed by submission of fees,* ***PRASENJIT MONDAL*** *vide* ***Enrolment No. PU/PHD/CHEM/2017/015*** *has been enrolled for the Ph.D. Programme of the Department of* ***CHEMISTRY,*** *Presidency University, Kolkata and course work starting from* ***SEPTEMBER, 2017.***

Head of the Department

Head
Department of Chemistry
Presidency University
Kolkata-700073

Dean of Faculty

Dean
Faculty of Natural and Mathematical Sciences
Presidency University, Kolkata

The Registrar

Registrar
Presidency University
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Date: 20 th Sept. 2017

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Grade Card –cum- Certificate

Certified that PRASENJIT MONDAL bearing Enrolment Number PU/PHD/CHEM/2017/015 of the Department of CHEMISTRY has successfully qualified the Ph.D. Coursework Examination in CHEMISTRY held in Odd Semester 2017- 2018, with the following grades.

Name: PRASENJIT MONDAL

Enrolment Number: PU/PHD/CHEM/2017/015

Paper	Credit	Grade obtained
Research Methodology	4	A+
Physical Chemistry of Polymer	4	A
Magneto-chemistry	4	A++
Review of Research Work	4	A+

Total Credits: 16

SGPA : 9.00

CGPA: 9.00

Date of Publication: 19TH February, 2018

Controller of Examinations

SL. NO. Science/S-63/19.

Presidency University

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REGISTRATION CERTIFICATE

This is to certify that Mr. Prasenjit Mondal, s/o Dipak Kumar Mondal, has been granted registration for the Ph.D. Programme in the **Department of Chemistry** of Presidency University, Kolkata in terms of the Regulations (No. 4) for the Degree of Doctor of Philosophy (Ph.D.).

Registration Number: **R-17RS205120098**

Date of Registration: **10th July, 2018**

Title of the thesis: "Investigation on the interaction of dietary phenolic acids and their derivatives with biologically significant molecules: A multi spectroscopic analysis"

This registration shall remain valid for five years with effect from the date of registration as indicated above.

Registrar

Date: 29th January 2019

Frontiers in Modern Biology (FIMB) 2018

Certificate of Participation

This is to certify that Dr./Mr./Ms. **Prasenjit Mondal**

has presented a poster in the Frontiers in Modern Biology (FIMB) 2018 organized by the Department of Biological Sciences, Indian Institute of Science Education and Research Kolkata, between January 19-21, 2018.

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We wish him/her a bright future ahead.

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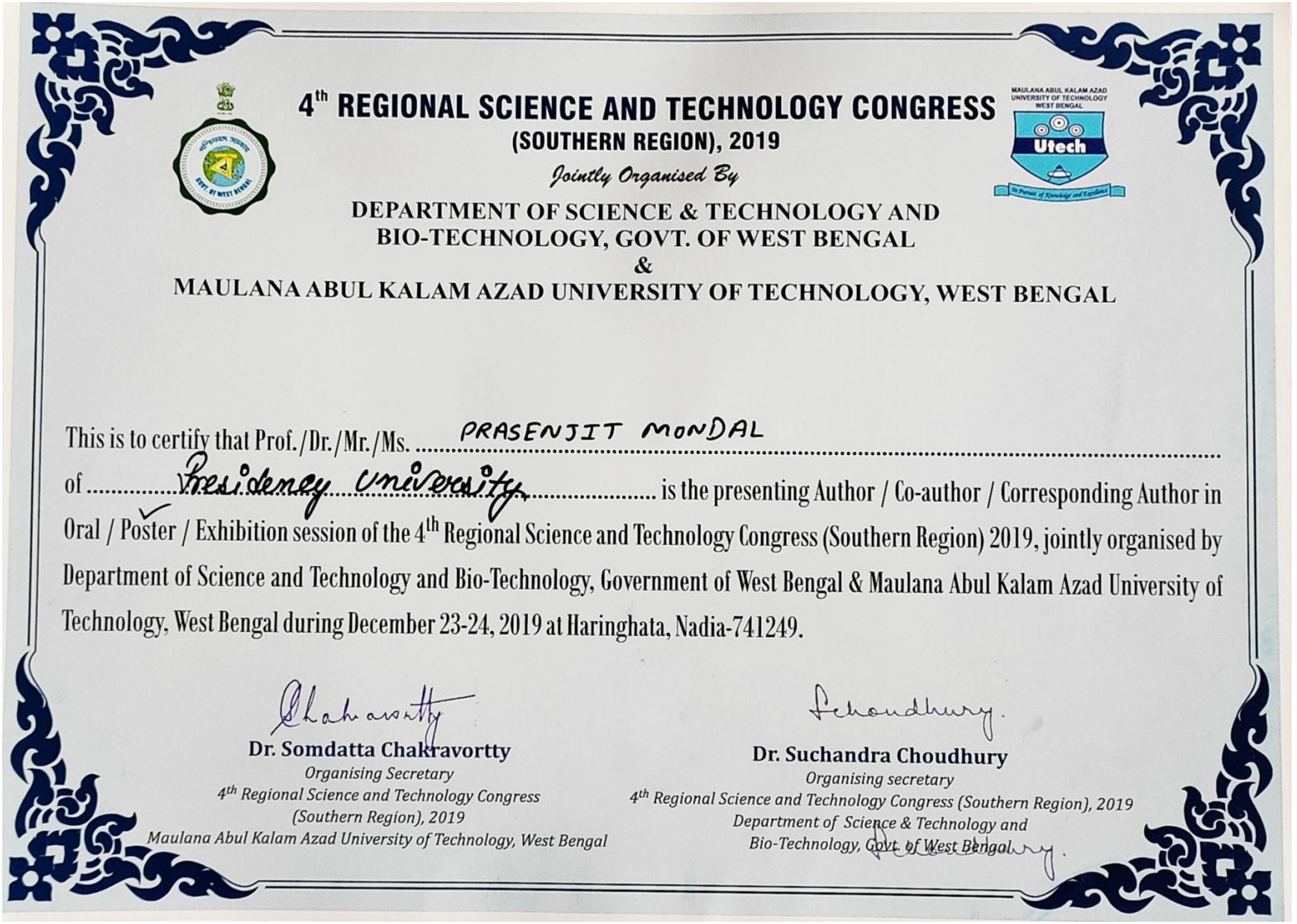

4th REGIONAL SCIENCE AND TECHNOLOGY CONGRESS
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This is to certify that Prof./Dr./Mr./Ms. PRASENJIT MONDAL of Presidency University is the presenting Author / Co-author / Corresponding Author in Oral / Poster / Exhibition session of the 4th Regional Science and Technology Congress (Southern Region) 2019, jointly organised by Department of Science and Technology and Bio-Technology, Government of West Bengal & Maulana Abul Kalam Azad University of Technology, West Bengal during December 23-24, 2019 at Haringhata, Nadia-741249.

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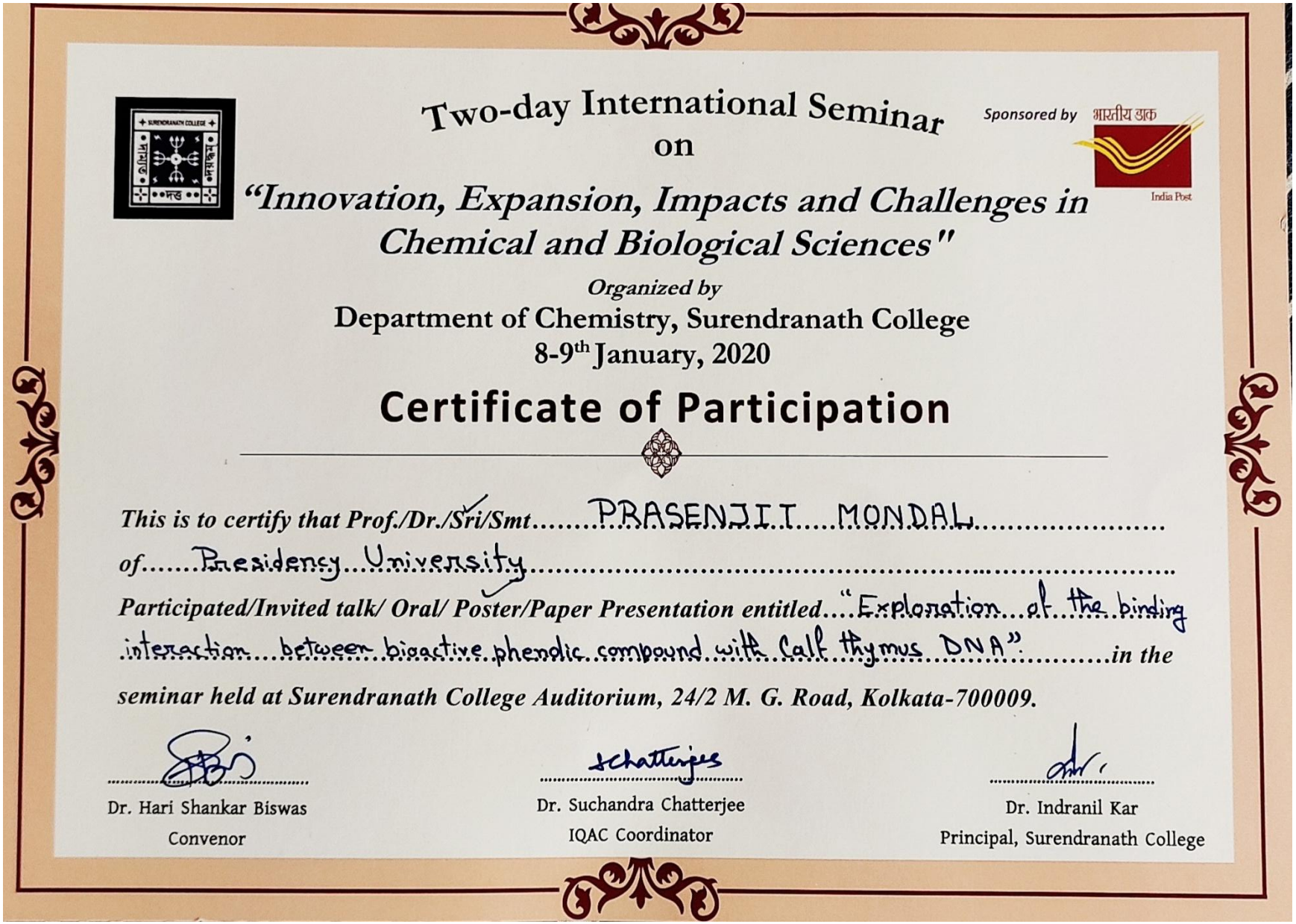

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Participated/Invited talk/ Oral/ Poster/Paper Presentation entitled.... "Exploration of the binding interaction between bioactive phenolic compound with Calf thymus DNA" .....*in the seminar held at Surendranath College Auditorium, 24/2 M. G. Road, Kolkata-700009.*

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Spectroscopic overview of quercetin and its Cu(II) complex interaction with serum albumins

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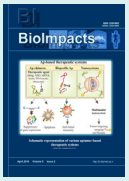

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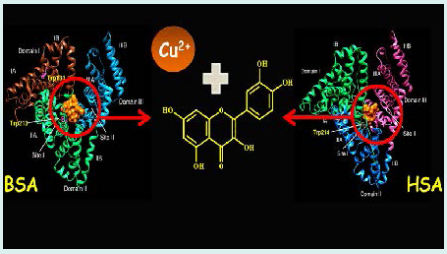
Abstract



Introduction: Flavonoids are widely used as dietary supplements, and thus, play a significant role in the research field. In recent time, the interaction of flavonoid-metal complexes with serum albumin (SA) has widely been studied since the complexation poses a significant impact on biological activities. Additionally, the binding nature of flavonoids with SA gets modified in the presence of metal ions.

Methods: In the present review, we studied the interaction of quercetin (Qu), a well-known flavonoid, and its Cu^{2+} complexes with SA to provide sufficient information about the beneficial role of metal-flavonoid complexes over free flavonoids.

Results: Complexation with Cu(II) ion may alter the mode of binding of Qu with SAs. The strength of binding might be increased in the presence of Cu(II) as evidenced by the binding constant calculation. However, the drug binding site in bovine serum albumin (BSA) and human serum albumin (HSA) are not altered during the complexation process.

Conclusion: To enhance the pharmaceutical outcomes of Qu molecules, one may use Qu-Cu(II) complex for the development and delivery of the small molecules.

Introduction

Flavonoids, a subgroup of polyphenolic compounds, are generated in plants, common fruits, vegetables, tea, wine, etc. In recent decades, flavonoids have widely been used in many biological activities, including cardiovascular diseases, cancers or age-related disorders.[1] Due to the variety of activities, the impacts of flavonoids have widely been considered on various biological processes.[2,3] Further, flavonoids can form chelates with the metal ions, which may change their antioxidant activity and biological effects.[4]

Quercetin (Qu), as shown in Fig. 1, is one of the most common bioactive and dietary flavonoids, which can be found in the flowers, leaves, and fruits of many plants.[5] Qu can form complexes with transition metal ions, such as Cu^{2+}, Mn^{2+}, Fe^{2+}, etc. Among them, the stability of Qu-Cu(II) complex is reported to be the highest.[5]

Cu(II) is one of the most abundant trace minerals in the human body. In the blood, it can often act as a cofactor for angiogenesis.[6] In some recent researches, it has been established that naturally occurring polyphenols can prevent the emergence of angiogenesis. Further, the catalytic role of transition metal ions such as Fe(III) and Cu(II) is well-known in the formation of hydroxyl radical (•OH) via metal-catalyzed Haber–Weiss reaction or Fenton reaction. Metal ion chelation by flavonoids is known to inhibit the aforementioned oxidative damage caused by metal ions. Also, the biological activities such as anti-oxidant effect, anti-bacterial and various kinds of enzymatic functions of free flavonoids get affected as a result of the complex formation.[7,8] Therefore, it is important to understand the effect of Cu(II) on the interactions between flavonoids and SAs. According to available literature, Qu-metal complexes seem to produce better biological activities than the bare Qu itself.[8,9]

In keeping with the above facts, in this review, we have studied the ultimate behavior of Qu and Qu-Cu(II) complex with SAs. SAs are the most abundant transport

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Biophysical and theoretical studies of the interaction between a bioactive compound 3,5-dimethoxy-4-hydroxycinnamic acid with calf thymus DNA

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ABSTRACT

3,5-Dimethoxy-4-hydroxycinnamic acid commonly known as Sinapic acid is a well-known derivative of hydroxycinnamic acids, is commonly present in human diet. Due to its wide variety of pharmacological activities like antioxidant, antimicrobial, anti-inflammatory, anticancer, and anti-anxiety, it has attracted much attention for the researchers. In our previous published work we have already analyzed the interaction between sinapic acid (SA) with a model transport protein. In this work our aim is to demonstrate a detailed investigation of the binding interaction between sinapic acid with another carrier of genetic information in a living cell, the DNA. Here we have used calf thymus DNA (ct-DNA) as a model. The binding characteristic of SA with ct-DNA was investigated by different spectroscopic and theoretical tools. The spectroscopic investigation revealed that quenching of intrinsic fluorescence of SA by ct-DNA occurs through dynamic quenching mechanism. The thermodynamic parameters established the involvement of hydrogen bonding and weak van der Waals forces in the interaction. Further, the circular dichroism, competitive binding experiment with ethidium bromide and potassium iodide quenching experiment suggested that SA possibly binds to the groove position of the ct-DNA. Finally, molecular docking analysis established the SA binds to minor groove position of ct-DNA in G-C rich region through hydrogen bonding interaction. Additionally, gel electrophoresis analysis has been performed to determine the protective efficacy of SA against UVB induced DNA damage and 50 μM of SA was found to protect the DNA from UVB induced damage. We hope that our study could provide the validation of SA on behalf of therapeutics and development of next generation therapeutic drug as well as designing new efficient drug molecule and methodology for the interaction study of the drug with DNA.

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1. Introduction

Study of the interaction of deoxyribonucleic acid (DNA) with small molecules is of immense interest in the arena of pharmaceutical chemistry [1]. DNA controls heredity of life in the living organism and involved in essential biological process such as mutagenesis, gene transcription, gene expression, carcinogenesis and cell death, that makes them interesting to many researchers [1–6]. According to literature the binding affinity and sequence specificity of small molecules with DNA depends on the various structural and electronic factors [1,7]. In this respect it is important to understand the binding affinities and binding mechanism between small molecules and DNA in modern clinical research.

Being a naturally occurring biologically significant molecule, phenolic acids are elaborately studied in the field of small molecule-DNA interaction. Phenolic acid contains a phenolic ring and organic carboxylic acid functionalities. Classes of naturally occurring phenolic acids are hydroxybenzoic acid and hydroxycinnamic acid, which are extensively found in nature, especially in vegetables, fruits and a variety of beverages like tea, coffee etc. The schematic diagram of phenolic acids and their application are summarized in Fig. 1. Due to a wide variety of pharmacological activity and availability of hydroxycinnamic acids, the primary interest of researchers is to study the nature and dynamics for DNA-drug binding, which can lead to the design and construction of new and more efficient drugs targeted to DNA. Very few researchers have investigated the interaction of DNA with hydroxycinnamic acids and its derivatives. The interaction of caffeic acid with ct-DNA was investigated by Sarwar et al. and they concluded that caffeic acid interacted with ct-DNA through groove binding [8]. Zhang et al. investigated the interaction of ferulic acid with calf thymus DNA and according

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Research Article

Stem Cell Research International

Evaluation of Sinapic Acid to Ameliorate Ionizing Radiation Induced Peripheral Blood Mononuclear Cell Death

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Abstract

Introduction*: The UV radiation is known as a potential environmental carcinogen which causes damages of macromolecules like DNA, RNA, lipid, and protein inside the cell and eventually causes cell death. Persons who chronically exposed to UV radiation due to occupational or environmental exposure have a potential risk to develop cancer of the skin and internal organs. Polyphenols have a promising role in scavenging reactive oxygen species developed in our body due to a variety of exposure including radiation. In the present study, we are therefore evaluating the role of sinapic acid (SA), a polyphenol, in scavenging UV radiation-induced generation of reactive oxygen species and eventual cell death. We have studied the biological activity of SA as a potential antioxidant on human peripheral blood mononuclear cells taken from human volunteers.*

Methods : *Graded concentration of SA in different solvent mediums was applied to UV exposed peripheral blood mononuclear cells for evaluating the best effective delivery system and concentration against UV radiation.*

Result*: After series of experiments, we have observed that even at 10 µM concentration, SA is effective against UV induced cellular death.*

Conclusion: *Finally, from this work we are inviting lots of works in this field to establish this molecule as a medicine to reduce radiation-induced cellular death.*

Keywords: Sinapic Acid, UVB Radiation, Antioxidant, Cytotoxicity, Peripheral Blood Mononuclear Cell.

Introduction

Ultraviolet radiation is a potent environmental carcinogen, induces oxidative and inflammatory skin damage and eventually cancer. Naturally occurring polyphenols are potent antioxidants and anti-inflammatory agents which can potentially reduce the damaging effects of ultraviolet (UV) rays on the human body. Reduction in the ozone layer in the atmosphere promotes considerable UV radiation to enter into the earth surface which may cause damages to the biological systems. Although both UVA and UVB are potentially harmful, the effect of UVB is more severe as it causes burn to the absorbing tissues. UV ray can penetrate the skin and contributes its damaging effects mostly on the keratinocyte and fibroblast. Most of the UVB is absorbed in the epidermis layer of the skin leaving little to enter into the dermis layer.

UVB induced damages most precisely causes cell death and apoptosis although not known to us that whether it may induce cell necrosis or not. Exposure to UVB causes formation of reactive oxygen species (ROS), cell cycle arrest, activation of various genes and cell markers [1]. Among all three types of UV rays, UVA and UVB rays can penetrate in human body. The UVA rays possessing the longest wavelength can reach deeper into the human skin, beneath the dermis layer, while, comparatively little UVB rays can penetrate into the dermis layer. UVC rays are fully absorbed by ozone layer and cannot reach the Earth surface. UVB is potentially the more dangerous than UVA rays and it causes burning of the cells and responsible for producing skin cancer.

The carcinogenicity of UVB is well established and has been understood as a potential damaging agent to DNA from which gene mutation arises. On the other hand, UVA is generally far more abundant on earth surface, and goes deeper into the skin surface,